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GENUS FRANCISELLA.(U)
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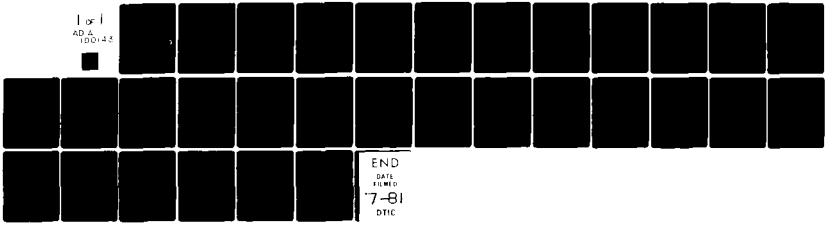

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A review of salient literature on the Genus, Francisella

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Genus Francisella Dorofe'ev 1947, 176

HENRY T. EIGELSBACH AND VIRGINIA G. McGANN

(Bacterium, McCoy and Chapin 1912, 61; Pasteurella, Bergey et al. 1923, 267; Brucella, Topley and Wilson 1929, 509; Francisella, Dorofe'ev 1947, 176).

Fran · cis · el'la. M. L. dim. ending -ella; M. L. fem. n. Francisella named for Edward Francis, an American bacteriologist who extensively studied the etiologic agent and pathogenesis of tularemia and is credited with naming the disease.

Relatively small, 0.2 - 0.7 μm by 0.2 μm, rod-shaped organisms when grown in appropriate media and examined during active growth; pleomorphism occurs subsequently. Faintly staining Gram negative. Capsule associated with virulence. Nonmotile. Obligate aerobe. On glucose cysteine blood agar smooth gray colonies reach maximal size in 2 - 4 days and are surrounded by a characteristic green discoloration. Catalase weak. Oxidase negative. Fermentation of small number of carbohydrates is characteristically slow with the production of acid but no gas. No growth on ordinary media without enrichment. Type species, Francisella tularensis, requires sulfhydryl compounds (cystine or cysteine) in media for growth. H_2S produced. Unlike other bacteria, F. tularensis contains relatively large amounts of long chain saturated and monoenoic C_{20} - C_{26} fatty acids as well as 3-hydroxy-hexadecanoate, 2-hydroxy-decanoate and 3-hydroxy-octadecanoate. These characteristics may represent a valuable taxonomic tool. The mol % G + C of the DNA is 33 - 36 (Bd; T_m).

15 May 1981

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Type species: Francisella tularensis (McCoy and Chapin) Dorofe'ev, 1947, 178; tu · la · ren'sis. M. L. adj. From Tulare County, California where the disease, tularemia, was first observed.

Further Descriptive Information

Morphologically Francisella tularensis and Francisella novicida are similar. When grown in appropriate liquid medium and examined during active growth, both are particularly small, singly occurring, nonmotile, nonsporulating, rod-shaped organisms; F. tularensis is slightly smaller, 0.2 - 0.7 μm long by 0.2 μm wide, while the size of F. novicida is 0.7 by 1.7 μm. Somewhat shorter forms of both occur in infected tissues. Generally, dye-stained clinical material is unsatisfactory for organism identification; application of direct or indirect fluorescent antibody techniques is recommended for rapid, specific recognition (Eigelsbach and McGann, 1981). In actively growing cultures Francisella appear as minute, faintly staining Gram negative, coccoidal organisms. On closer inspection, however, these forms are visualized as bipolar components of a rod-shaped organism separated by an even more faintly stained central area enclosed by a delicate cell wall. This characteristic morphology is more readily demonstrable with a polychrome (Giemsa) stain. During the logarithmic growth phase in a liquid medium shaken at 37°C, cells are relatively uniform; soon thereafter, pleomorphism is observed. During the phase of decline (after 24 h incubation), the cell wall elongates, filamented cells form and filament fragmentation frequently occurs; also, at this time, a small number of rods not larger than 300 nm in diameter can be visualized by electron microscopy (Ribi and Shepard, 1955). Minute forms, infectious for mice, pass through membranes with 600 nm average

pore diameter and are estimated to be in the range of 300 - 350 nm in diameter (Foshay and Hesselbrock, 1945). These authors found no morphological feature to differentiate a virulent from a nonvirulent strain.

Divergent views exist concerning the reproduction of F. tularensis. Hesselbrock and Foshay (1945) using light microscopy described multiple modes of reproduction in gelatin-hydrolysate liquid medium. Budding was observed frequently and appeared to be the chief mode of reproduction; binary fission, though not seen, was still considered a possibility. Many morphological units including "minimal reproductive units" were described. Electron microscopic studies (Ribi and Shepard, 1955) performed with organisms taken from actively growing cultures revealed cells with constrictions suggesting multiplication by binary fission. During the death phase filamented cells easily fractured into pieces corresponding to the forms reported by Hesselbrock and Foshay (1945). Ribi and Shepard (1955) concluded that the possession of a complex life cycle is unlikely with regard to F. tularensis. Excellent electron micrographs of F. tularensis (Hood, 1977) provide additional evidence of reproduction by binary fission.

Virulent organisms of F. tularensis are surrounded by a relatively thick (0.02 - 0.04 μm), electron transparent capsule that is removed quite easily in solutions containing sodium chloride; loss of capsule is accompanied by loss of virulence but viability is unaffected (Hood, 1977). With loss of capsule the organisms become less resistant to acid pH levels that simulate the lysosomal environment of infected macrophages (Canonico, P. G., 1981, unpublished results). In material from infected animals, stained organisms are found to be surrounded by a clear area presumed to be capsule, and if virulent organisms from

culture are mixed with serum, capsules may be demonstrated. Underlying the capsule is the delicate, double-layered cell wall that is easily distorted during cytological processing. Lipid concentration in capsule and cell wall, 50% and 70% respectively, is unusually high for Gram negative bacteria; the lipid composition is also characteristic: saturated straight-chain 16:0 (48%) and α-OH 14:0 (31%) fatty acids in the capsule as compared with the cell wall that has only a trace of α-OH 14:0 but contains principally β-OH 10:0 (30%) with six other fatty acids (60%) in approximately equal proportions. Capsule and cell wall differ quantitatively and qualitatively in sugar composition, but amino acid analysis suggests similarity in structure of their protein moiety with a difference in the amount present, 35% total amino acids in the capsule and 8% in the cell wall (Hood, 1977).

On glucose cysteine blood agar (GCBA) and peptone cysteine agar (PCA) viscous, readily emulsifiable colonies reach maximal size after 2 - 4 days at 37°C, 1 - 4 mm for F. tularensis and 6 - 8 mm for F. novicida; growth of F. novicida is usually more rapid and luxuriant than that of F. tularensis. On GCBA medium colonies are surrounded by a characteristic green discoloration not associated with true hemolysis; on "chocolatized" agar the discoloration is brownish in appearance. Colony type variants, including those associated with virulence and immunogenicity, are best detected when observed on transparent agar illuminated by obliquely transmitted light (Fig. 1) (Eigelsbach et al., 1951, 1952; Eigelsbach and Downs, 1961).

Francisella are strict aerobes and grow optimally at 37°C. F. tularensis grows much more slowly and less abundantly than F. novicida (Larson et al., 1955; Owen et al., 1964). F. novicida is less fastidious than is F. tularensis and does not require addition of a

sulfhydryl compound for cultivation but growth is markedly enhanced by addition of cysteine or cystine to media. Unlike F. tularensis, F. novicida grows moderately well with uniform turbidity in proteose peptone broth without cystine and somewhat less abundantly in nutrient gelatin without causing liquefaction (Owen et al., 1964).

F. tularensis cannot be cultured routinely on conventional laboratory media, but two commercially produced solid media, glucose cysteine agar (GCA) with thiamine[a] or cystine heart agar[b] (when either is enriched with defibrinated rabbit blood or outdated, settled, human blood cells) provide excellent growth from small inocula. Another solid

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[a]GCA agar with thiamine (BBL, Bio Quest). When used with added blood the medium is commonly referred to as GBCA and can be substituted for the original, noncommercial medium described by Downs et al., 1947. Suspend 58 g of the dry material in a liter of distilled or demineralized water. Mix thoroughly. Heat with frequent agitation and boil for 1 min. Dispense and sterilize tubes by autoclaving at 118 to 121°C, for 15 min. For liter quantities, autoclave at the same temperatures for 30 min. Cool to 45 - 48°C. Aseptically add 25 ml of packed, human blood cells or 50 ml of defibrinated rabbit or sheep blood. Mix thoroughly and pour into plates. Incubate at 37°C for 24 h before use to decrease surface moisture and test for sterility.

[b]Cystine Heart Agar (Difco). Suspend 102 g of the dry material in a liter of distilled or demineralized water. Follow procedure indicated in "a" above.

medium[a] containing blood, equally satisfactory but not produced commercially, has been described (Gaspar et al., 1961). A transparent medium such as PCA[b] (Snyder et al., 1946) is required for the detection of colony type variants described in the literature.

Each lot of commercial medium and the protein-derived ingredients in the other media described should be quality-control tested for the ability to support satisfactory growth of avirulent F. tularensis ATCC 6223 or live vaccine strain LVS (Eigelsbach and Downs, 1961). Occasionally a marginal lot of medium will yield more rapid and abundant growth upon addition of 0.5 g ferrous sulfate per liter of medium. Incorporation of antibiotics (penicillin, polymyxin B and cycloheximide) is required when clinical specimens containing normal flora are cultured for F. tularensis.

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[a]Francisella tularensis Isolation Medium. Number g/liter distilled or demineralized water: tryptose broth with thiamine (Difco), 20; cysteine HCl, 5; sodium thioglycolate, 2; glucose, 10; agar, 10. Mix all ingredients except agar and adjust pH to 7.2. Add agar and heat in flowing steam for 5 min. Autoclave at 121°C for 20 min. Cool to 45 - 48°C. Aseptically add 50 ml defibrinated rabbit blood. Mix thoroughly and pour into plates. Incubate at 37°C for 24 h before use to decrease surface moisture and test for sterility.

[b]PCA medium. Number g/liter distilled or demineralized water: peptone (Bacto), 20; NaCl, 10; glucose, 1; cysteine HCl, 1; agar, 20. Mix all ingredients except agar and adjust pH to 6.8. Add agar. Heat with agitation and boil for 1 min. Cool to 45 - 48°C. Pour into plates and incubate at 37°C for 24 h before use to decrease surface moisture and test for sterility.

Satisfactory liquid media for the cultivation of F. tularensis include: peptone cysteine broth (Snyder et al., 1946); casein partial hydrolysate (Mills et al., 1949); and several chemically defined media (Mager et al., 1954; Traub et al., 1955; Nagle et al., 1960; and Chamberlain, 1965). Continuous shaking for 12 - 18 h during incubation at 37°C is required for maximal growth (10×10^9 - 40×10^9 viable organisms per ml).

Under suitable conditions, anaerogenic fermentation of a limited number of carbohydrates occurs with slight acid production; slow development of minimal reactions is characteristic of F. tularensis. All strains ferment glucose, fructose and mannose; maltose fermentation is commonly seen with F. tularensis and sucrose fermentation is a distinguishing feature of F. novicida (Girard and Gallut, 1957). Inability of F. tularensis var. palaearctica to utilize glycerol assists differentiation of these organisms (Olsufiev et al., 1959). A third proposed variety, var. mediaasiatica now included in var. palaearctica, is reported to ferment glycerol but not maltose (Kunitsa et al., 1972). Growth in litmus milk is scant; slight acidity may develop in F. tularensis cultures in 2 weeks and an acid reaction with soft coagulation in F. novicida cultures (Owen et al., 1964). Possession of a citrulline ureidase system is an identifying characteristic for F. tularensis var. tularensis (Marchette and Nicholes, 1961). Catalase activity is weak but strongest in strains of F. tularensis var. tularensis; attenuation of virulence is accompanied by a marked reduction in activity (Rodionova, 1976). Strains of F. tularensis var. tularensis contain cytochromes a_1, a_2 and b_1 but no a_3 or c-type cytochromes or cytochrome oxidase (Fellman and Mills, 1960); the amount of cytochrome b_1 in the whole cell and cell-free extract is reported to be greater for virulent

than for avirulent organisms (Mizuhara and Yamanaka, 1961). An active succinoxidase system in F. tularensis var. tularensis is located in the particulate fraction of the cell and interacts with cytochrome b_1 (Fellman and Mills, 1960).

Hill (1966) reported that values for deoxyribonucleic acid base composition of F. tularensis, 33 - 36 mol % GC, are markedly lower than those for Brucella abortus, Yersinia pestis or coliforms. The hybridization experiments of Ritter and Gerloff (1966) reveal a distinct reciprocal relationship between DNA of F. tularensis and F. novicida (more than 78% of ^{32}P-labeled DNA bound by the heterologous type) and essentially no hybridization between DNA from Francisella and that of Yersinia or Pasteurella. Transformation and recombination studies within the genus Francisella are in accord with these data (Tyeryar and Lawton, 1969, 1970). Koliaditskaia et al. (1959) described lytic activity in F. tularensis cultures consistent with that expected of a phage. However, no phage for F. tularensis or F. novicida has been characterized or is available for use.

The antigenic composition of F. tularensis has been examined primarily for detection of infection and identification of the infectious organism or of nonviable immunogenic substances. Conventional serologic procedures, including direct and indirect immunofluorescent techniques, indicate that all F. tularensis are qualitatively similar in antigenic composition, and convalescent sera or immune sera produced with whole organisms react with and can be absorbed by all strains. Live vaccine is a highly effective immunogen against infection of susceptible hosts with fully virulent strains (Eigelsbach and Downs, 1961; Saslaw et al., 1961; McCrumb, 1961; Hornick et al., 1966; Hornick and Eigelsbach, 1966); nonviable vaccines induce similar protection only

in resistant hosts or against strains of moderate virulence in the susceptible host.

Antigenic material has been extracted from F. tularensis with phenol, acetone, water saturated with ether, or trichloroacetic acid (Nicholes, 1946; Ormsbee and Larson, 1955; Nutter, 1971; Hambleton et al., 1974; Holm et al., 1980). Chemical analysis and electron microscopy suggest that these preparations lack nucleic acid and are derived from the cell wall. All products contain multiple antigenic factors, induce agglutinins and/or precipitins, are nontoxic and have immunogenic activity similar to that of killed whole cells. Other extracts, rich in RNA, have similar immunogenic properties but their activity is destroyed by RNase (Andron and Eigelsbach, 1975). Capsular material is neither immunogenic nor toxic (Hood, 1977). One type of nonviable preparation, a suspension of cells killed by gamma-irradiation, protects mice against infection with fully virulent F. tularensis but the immunogenic activity disappears within 4 days after irradiation (Gordon et al., 1964). This preparation has two toxic components lethal for mice, guinea pigs and rabbits: the activity of one is labile at 4°C, declining with the decrease in immunogenicity, whereas that of the other is stable and has endotoxin-like properties (Landay et al., 1968). Immune or convalescent sera can protect mice against the toxic activities but are ineffective against infection.

F. tularensis has a minor serological relationship to Brucella (Saslaw and Carlisle, 1961) and Y. pestis (Larson et al., 1951). A closer relationship is seen with F. novicida (Owen et al., 1964). Live F. tularensis vaccine protects against both species, while live F. novicida vaccine protects against homologous infection and against F. tularensis strains of moderate virulence. Nonliving vaccines protect

only against homologous organisms. Cross-reactions between Francisella species occur in complement fixation tests with guinea pig antisera, but agglutination, hemagglutination, and passive cutaneous anaphylaxis reactions are highly specific. Nonspecific induction of protection against F. novicida but not against F. tularensis has followed immunization with BCG vaccine (Claflin and Larson, 1972).

The aminoglycosides, streptomycin, gentamycin, and kanamycin are bactericidal for F. tularensis; whereas, the tetracyclines and chloramphenicol are bacteriostatic. Streptomycin is preferable for the treatment of severe disease including pneumonic tularemia. Although gentamycin and kanamycin are highly effective in these illnesses, experience with these antibiotics is meager and greater risk of oto- and nephrotoxicity is incurred (Woodward, 1979; Hoover, 1979). Because of their bactericidal nature, these aminoglycosides are not associated with disease relapse but the drugs must be administered parenterally.

Less severe tularemia (ulceroglandular without secondary pneumonia) is usually treated by oral administration of tetracycline. Relapses with this bacteriostatic drug are more frequent when therapy is initiated during the first week of the disease than 10 to 12 days after onset of illness. Retreatment with the same antibiotic is followed by a prompt response. Choramphenicol is as effective as tetracycline for treatment of less severe tularemia but is not recommended because of potential hematologic toxicity. Antibiotic-resistant strains have been induced in vitro but have never been isolated in cases of naturally occurring tularemia.

A combination of penicillin, polymyxin B and cycloheximide is added to any of the recommended media used to isolate F. tularensis from clinical specimens expected to contain normal flora. Occasionally

ordinary media inoculated with infected tissue will initially support growth of F. tularensis. The addition of colistin, mystatin, lincomycin and trimethroprim to chocolate agar routinely used for the isolation of gonococci has been reported to allow also the isolation of F. tularensis from some experimental mixtures containing flora prepared from human throat cultures (Berdal and Soderlund, 1977). Routine susceptibility testing is accomplished with the disk agar diffusion method. Reading are made after overnight incubation at 37°C.

F. tularensis is widely distributed in nature and is found on all continents throughout the world except Australia and Antarctica. The two main regions in which numerous infections of humans have occurred over a relatively large area are the USA (all states except Hawaii) and southern USSR. Two varieties are recognized and differ in virulence. One appears only in North America, where it is predominant and highly virulent for numerous hosts. The other is found in Europe and Asia as well as in North America but causes a milder disease in humans and domestic rabbits. The principal reservoirs for this type are voles and water rats in the USSR and beavers, muskrats and voles in North America. The organism can be isolated from natural waters (streams, rivers, lakes or ponds) in areas frequented by these animals. F. tularensis has been isolated from approximately 100 types of wildlife, about one-half of which have transmitted tularemia to man. They include wild rabbits, muskrats, water rats, beavers, squirrels, woodchucks, sheep, mice, voles and game birds as well as biting insects (usually ticks or deer flies). Infection follows handling of infected animal carcasses, insect bites, ingestion of improperly cooked meat or contaminated water, or inhalation of airborne organisms, especially during processing of agricultural products contaminated by infected rodent excreta. Bites or scratches by

resistant wild or domestic carnivores (dog, cat, skunk, coyote, fox, hog or bull snake) whose mouth parts have been contaminated by eating infected animals may also result in human infection. F. tularensis has great invasive ability and is able to penetrate the unbroken skin. Although humans of all ages, sexes and races are susceptible, man-to-man transmission is extremely rare.

Human tularemia is an acute, febrile, granulomatous, infectious, zoonotic disease. During evaluation of vaccine prophylaxis in volunteers it was determined that nonvaccinated individuals could be infected subcutaneously with 10 organisms or by inhalation of 10 - 50 cells (Saslaw et al., 1961). The clinical picture and severity vary appreciably according to the route of infection and the virulence of the organism. The incubation period in humans is usually 3 to 4 days but ranges from 2 to 10 days dependent primarily on dose.

Before the advent of antibiotic therapy, tularemia in untreated patients in North America resulted in a case mortality rate of approximately 5 - 30%. In Eurasia mortality rates were lower for all untreated clinical types (averaging less than 1%) because of inherently lower virulence of the variety common to that area.

In general, F. novicida is less virulent than F. tularensis. It is experimentally pathogenic for white mice, guinea pigs and hamsters producing lesions similar to those of tularemia, while rabbits, white rats and pigeons are resistant (Owen, 1974). Human infections have not been reported.

Enrichment and Isolation

Detailed procedures for the collection, transport and storage of clinical specimens have been described by Eigelsbach (1974). Procedures

recommended for isolation, described in detail for F. tularensis (Eigelsbach and McGann, 1981), are equally satisfactory for F. novicida. Impinged air samples or water from suspected streams or wells are passed through 0.45-μm cellulose acetate filters and the trapped organisms are freed by vigorous agitation of the filter in sterile saline containing 0.1% gelatin. The volume of the inoculum of these and other liquid materials should not exceed 0.2 - 0.3 ml per plate of recommended media. When enriched with blood, commercially prepared GCA with thiamine or cystine heart agar is satisfactory for isolation from most air, water or clinical specimens. Incorporation of antibiotics into the medium (penicillin, 100,000 units/ml; polymyxin B sulfate, 100,000 units/ml and cycloheximide, 0.1 mg/ml) is recommended when specimens are expected to contain abundant normal flora. It is essential that laboratory personnel utilize a syringe (needle removed) or a pipette fitted with a safety suction device to transfer potentially highly infectious material to the medium. Smeared or streaked plates are inverted and incubated at 35 - 35°C for 48 - 96 h. Increased CO_2 is not required, but is not harmful.

Direct culture has the advantage of being more rapid and less hazardous than inoculation and housing of infected animals but it must be anticipated that this procedure might fail. A portion of all specimens should be held at -30 to -70°C for further reference. Procedures for isolation by inoculation of laboratory animals and examination of infected tissues, including laboratory and animal room safety requirements, have been detailed by Eigelsbach (1974).

Maintenance Procedures

Repeated serial transfer of F. tularensis in liquid or on solid media routinely leads to a loss in virulence of the culture. Solid media recommended for isolation and cultivation are satisfactory for limited subculturing. Viability and virulence remain constant for years if 12 - 18 h growth is harvested from liquid or solid medium and frozen or lyoprocessed with equal volumes of skim milk or 20% sucrose, 2.6% gelatin and 0.2% agar (Faibich, 1959; Faibich and Tamarkina, 1946). Frozen cultures are stored at -30 to -70°C and lyoprocessed preparations are held at 5 to -70°C. F. novicida remains viable and virulent when lyophilized in skim milk and stored under vacuum at 4°C.

Differentiation of the Genus Francisella from Other Genera

Table 1 indicates the characteristics of Francisella that can be used to distinguish this genus from other genera of small, Gram negative, nonsporing coccobacillary bacteria that are pathogenic for man and other mammals.

Taxonomic Comments

The taxonomic position of the genus Francisella remains uncertain as indicated in the previous edition of this Manual. The highly infectious nature of these bacteria is one of the reasons responsible for slow development of critical evidence for taxonomic classification. The following information is derived from studies with a limited number of "representative" strains. DNA hybridization indicates that the genus is not closely related to Pasteurella, Yersinia or the coliforms (Ritter and Gerloff, 1966), and the mol % G + C in DNA is significantly lower than that of Brucella or Yersinia. In addition, the cellular fatty acid

composition of Francisella is distinctly different from that of other Gram negative bacteria, including Brucella, Pasteurella and Yersinia (Jantzen et al., 1979), and the unusually high lipid content of the cell wall is unlike that of other Gram negative bacteria (Hood, 1977).

A close relationship between F. tularensis and F. novicida is indicated by DNA hybridization studies (Ritter and Gerloff, 1966) and by demonstration of equally high transformation frequencies in F. novicida organisms treated with homologous or with F. tularensis DNA (Tyeryar and Lawton, 1970). The species F. novicida, consisting of a single known isolate (ATCC 15482) apparently avirulent for man, has less fastidious metabolic requirements than F. tularensis; these metabolic as well as serologic differences support designation of a separate species because loss of virulence in F. tularensis generally is accompanied by a requirement for more exacting growth conditions.

Olsufiev et al. (1959) recognized two major tularemia pathogens and recommended the designation Francisella tularensis var. tularensis for the organism prevalent in North American and responsible for the higher incidence and greater severity of human illness there, and Francisella tularensis var. palaearctica for the organism encountered in Europe, Asia and the Americas. F. tularensis var. tularensis is associated with tick-bourne tularemia in rabbits, produces the classical illness described in most medical texts and is highly virulent for man; F. tularensis var. palaearctica is frequently linked with water-bourne disease of rodents in North America and Eurasia and causes a milder form of illness. In North America the two kinds of tularemia are characterized by epidemiological pattern and virulence of the etiologic agent; tentative designations of type "A" and type "B" have been proposed for the more virulent and less virulent types (Jellison, 1974).

Since 1970, investigators in the USSR have used the designations F. tularensis nearctica Olsufiev for F. tularensis var. tularensis and F. tularensis holarctica Olsufiev for F. tularensis var. palaearctica. Additional subdivisions of the latter group are designated F. tularensis holarctica var. japonica Rodionova for Japanese-type strains and F. tularensis mediaasiatica Aikimbaev for strains from Central Asia. Additional information may be found in a review of tularemia in North America from 1930 to 1974 (Jellison, 1974) and in a review of investigations in the USSR (Pollitzer, 1967).

Differentiation and Characteristics of Species of Francisella

Differential characteristics of the species of Francisella are presented in Table 2 and other characteristics of the species in Table 3.

List of the Species of the Genus Francisella

1a. Francisella tularensis var tularensis Olsufiev et al., 1959, 146.

Morphology and characteristics are as for the genus and as in Tables 1, 2 and 3.

Wild strains are highly infectious and virulent for man; they are as virulent for the domestic rabbit as for the white mouse or guinea pig. Spontaneous loss of virulence can occur when strains are maintained on artificial media.

Found in nature only in North America, particularly in lagomorphs and wild rodents. May be transmitted by the bite of ticks or deer flies or by contact with, or ingestion of, contaminated animal carcasses.

Type strain: ATCC 6223 (strain B38, an avirulent strain with more fastidious growth requirements and greater tendency toward variation in colony type than virulent strains). Virulent strains, ex. SCHU, are available only from individual investigators.

1b. Francisella tularensis var. palaearctica Olsufiev et al., 1959, 148.

pa · lae · arc'ti · ca. M. L. adj. palaearctica of the Old World, Northern Hemisphere.

Wild strains cause milder human illness than F. tularensis var. tularensis. At least 10^5 more organisms are required to kill the domestic rabbit than to kill the white mouse or guinea pig.

Found in nature, wherever tularemia occurs, and is associated with a variety of wild rodents. Present in natural waters or in agricultural products contaminated by these animals.

Type strain: None available at ATCC. The avirulent live vaccine strain can be obtained by consultation with the Centers for Disease Control, Attention: Immunobiologics Activity, Atlanta, GA 30333, USA. Virulent strains are available from individual investigators.

2. Francisella novicida (Larson et al.) Olsufiev et al., 1959, 146. (Pasteurella novicida Larson et al., 1955, 253)

no · vi'ci · da. L. adj. novus new; L. v. suff. -cida from L. v. caedo cut, kill; M. L. n. novicida new killer.

Characteristics and metabolism as described for genus and in Tables 1, 2 and 3. Coccoid to ovoid or short rod-shaped cells, 0.2 - 0.3 µm by 0.3 µm in tissues, 0.7 by 1.7 µm in liquid media and 0.5 µm by 0.5 - 0.9 µm on solid media. Capsules not observed.

Methyl red test negative; acetylmethylcarbinol and indole not produced. Nitrate not reduced to nitrite. Methylene blue reduced.

Produces lesions similar to tularemia in white mice, guinea pigs and hamsters, but virulence is lower than that of most strains of F. tularensis. Rabbits, white rats and pigeons are resistant. Not known to infect man.

Isolated from a water sample taken from Ogden Bay, Utah, 1951.

Type strain: ATCC 15482.

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TABLE 1

Differential characteristics of the genus Francisella and other genera of small, Gram negative, nonsporing, coccobacillary bacteria

Characteristic	Francisella	Brucella	Pasteurella	Yersinia
Cells: $\leq$ 0.25 µm x $\leq$ 0.5 µm	+	-	D	-
Capsule easily demonstrated	-	-	+	+
Gram stain: weak counterstain	+	+	-	-
Strict aerobe	+	+	-	-
Optimal temperature 37°C	+	+	+	-
CO_2 enhances growth	-	+	+	-
Cysteine/cystine requirement	+	-	-	-
Chain forms in liquid culture	-	+	+	+
Carbohydrate fermentation[a]:	+[b]	-	+	+
Sucrose	-	-	+	D
Catalase	+[b]	+	+	+
Oxidase	-	D	+	-
NH_3	-	+	+[b]	+
Sodium ricinoleate solubility	+	-	+	+
Penicillin-sensitive in vitro	-	-	D	D
Insect vectors	+	-	+	+
Francisella antisera reactions				
Agglutinin	+	D	-	D
Fluorescent antibody	+	-	-	-
Francisella antibodies absorbed	+	-	-	-
Mol % G + C of DNA	33 - 36	56 - 58	36 - 43	46 - 47

[a] Anaerogenic fermentation

[b] Weak reaction

TABLE 2

Differential characteristics of Francisella tularensis and Francisella novicida

Characteristic	1a. F. tularensis var. tularensis	1b. F. tularensis var. palaearctica	2. F. novicida
Capsule	v[a]	v[a]	-
Growth requirement:			
Cystine or cysteine	+	+	-
Growth media:			
blood agar, gelatin, peptone broth	-	-	+
Colonies on GCBA[b] > 5 mm	-	-	+
Acid produced from: Maltose	+	+[c]	-
Sucrose	-	-	+
Glycerol	+	-[d]	+
Citrulline ureidase	+	-	
Serum agglutination:			
F. tularensis	+	+	-
F. novicida	-	-	+
Vaccine efficacy:			
Killed vaccine			
F. tularensis	+[e]	+[e]	-
F. novicida	-	-	+
Live vaccine			
F. tularensis	+	+	+
F. novicida	+[e]	+[e]	+

[a]Capsule associated with virulence of the strain

[b]Glucose cystine blood agar

[c]Central Asian strains (proposed variety mediaasiatica) reported to be negative

[d]Central Asian strains (proposed variety mediaasiatica) reported to be positive

[e]Protects fully susceptible hosts only against strains of partially reduced virulence

TABLE 3

Other Characteristics of Francisella tularensis and Francisella novicida

Characteristic	1a. F. tularensis var. tularensis	1b. F. tularensis var. palaearctica	2. F. novicida
Pleomorphic after logarithmic growth	+	+	+
Bipolar staining (Giemsa)	+	+	+
H_2S from cysteine/cystine	+	+	+
Motility	-	-	-
NH_3	-	-	-
Gelatin hydrolysis	-	-	-
Indole	-	-	-
Litmus milk after 2-week incubation:			
Slight acidity	+	+	+
Soft coagulation	-	-	+
Acid from carbohydrates[a]:			
Glucose, fructose, mannose	+	+[b]	+
Median sc infectious dose $< 10^3$ organisms:			
Mice, guinea pigs	+[c]	+[c]	+
Domestic rabbits	+[c]	-	-
White rats	-	-	-
Humans	+[c]	+	-

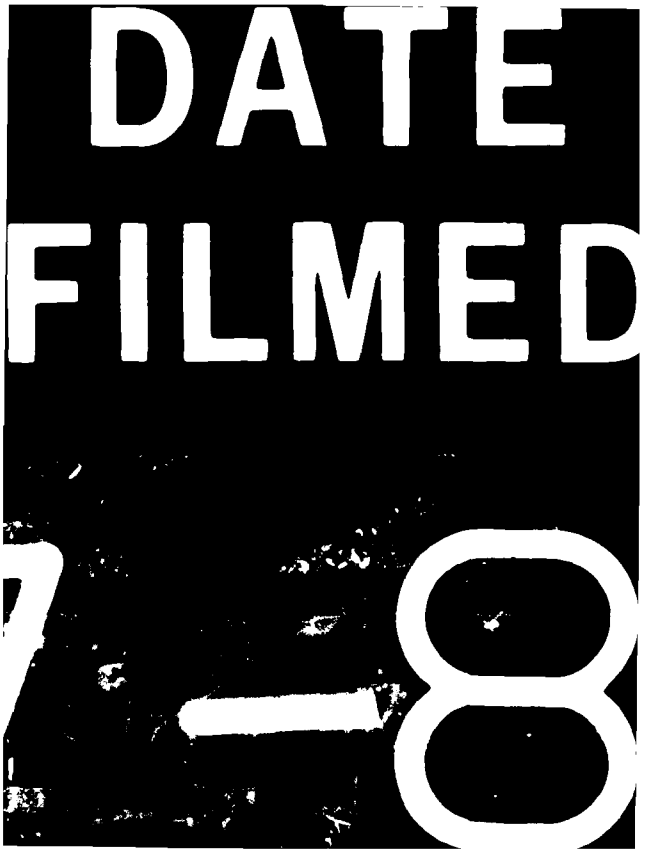
[a]Slight acidity develops slowly in media with cysteine or cystine

[b]Central Asian strains (proposed variety mediaasiatica) reported to be negative

[c]Median infectious dose, 1 - 10 organisms; this dose is lethal for mice, guinea pigs and domestic rabbits.

FIGURE LEGEND

Fig. 1. Colony type variants of Francisella tularensis on peptone cysteine agar, X 100. (Reproduced by permission of The Williams and Wilkins Company, Baltimore, MD, from: H. T. Eigelsbach, W. Braun and R. H. Herring, J. Bacteriol. 61:557-569, 1951).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